In Vivo Effects of Chimeric Oligonucleotides

Methods: Human U-87 glioblastoma cells were implanted subcutaneously into the flank region of athymic BALB/c nu/nu mice where they grow into palpable tumor within 3 weeks. Animals carrying tumors of similiar size were divided into six groups of 4 mice each and were injected intraperitoneally with one dose of 8 mg/kg of an oligonucleotide (see below) dissolved in 0.1 ml PBS, or PBS alone. 48 h later the mice were killed and the tumors removed, washed in PBS, and about 100 mg of the tumor were homogenized at 4°C in 200 µl of CHAPS-lysis buffer and centrifuged at 12000 g for 30 min at 4°C. 2 µl of tumor extract were added to the TRAP assay (Intergen; Purchase, NY) to estimate the telomerase activity as described (Matthes and Lehmann, Nucleic Acids Res.1999, 27: 1152-1158): The enzyme activity was related to the protein concentration.

As described in the patent application the sequence of the chimeric oligonucleotides consists of a phosphorothioate (PS)-modified part at the 5'-end with a random sequence (10mer/PS, red characters) for the binding to the primer bindung site of telomerase protein and an antisense part (T11, blue characters) which binds to the complementary sequence of the template region of the telomerase RNA as demonstrated in the Figure. We used 5'-d(ACTGCTCAGA--GTTAGGGTTAG) The antisense sequence was modified by N3'→P5' phosphoramidates (PAM) or by 2'-O-methylribonucleosides (Ome). Furthermore we investigated the following oligonucleotides as controls: 5'-d(ACTGCTCAGA) and 5'-d(ACTGCTCAGA-TCAGATA CAGA), a 10mer PS-ODN and a chimeric ODN in which the antisense sequence part was replaced by a nonsense sequence (11n, purple characters).

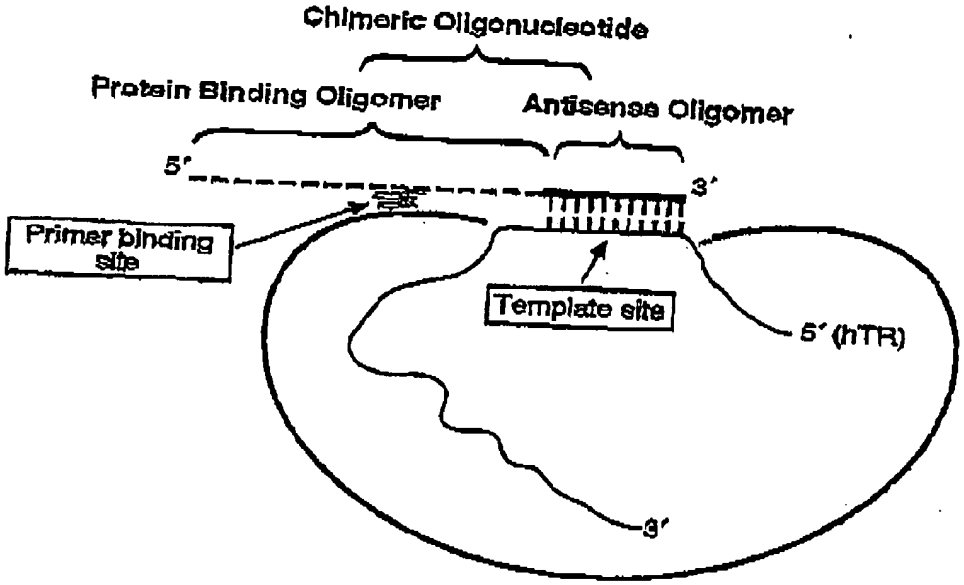

Figure Model of the human telomerase with the primer binding site of the protein and the template site of the RNA which both are targeted by our chimeric oligonucleotides

Results. The table summarizes the first results obtained. It can be seen that chimeric ODNs display an inhibitory effect on telomerase activity in a human U-87 glioblastoma tumor in nude mice 48 h after injection (8 mg/kg). Most effective chimeric ODN was 10mer/PS/T11/PAM which inhibited telomerase activity to 50-75%. A chimeric ODN with the same sequence and number of PS-linkages in which the PAM-modification of the antisense part was replaced, however, by the 2'-Ome-modification gave 40-64% inhibition of telomerase activity (10mer/PS/T11/Ome). In contrast control ODNs such as the pure antisense ODN (T11/PAM) were much less effective (11-25% inhibition) or inactive (10mer/PS and 10mer/PS/11n/PAM; 3-6% inhibition).
Therefore, it can be concluded that effective concentrations of chimeric ODNs can reach the human U-87 tumor in mice, enter the glioblastoma cells and display antitelomerase activity.

Table
Influence of a single i. p. injection of ODNs (8 mg/kg) on telomerase activity of human U-87 glioblastoma tumors grown in nude mice.

Oligonucleotide applied	% Telomerase activity remaining 48 h after injection
No	100%
5'-d(ACTGCTCAGA-GTTAGGGTTAG) 10mer/PS/T11/PAM	25-50%
5'-d(ACTGCTCAGA-GTTAGGGTTAG) 10mer/PS/T11/Ome	36-60%
5'-d(ACTGCTCAGA) 10mer/PS	95-97%
5'-d(GTTAGGGTTAG) T11/PAM	75-89%
5'-d(ACTGCTCAGA-TCAGATACAGA) 10mer/PS/11n/PAM	94-96%

Abbreviations: PS = phosphorothioate; PAM = N3'→P5' phosphoramidate; Ome = 2'-O-methylribonucleoside; T11=sequence complementary to the template region of telomerase RNA; 11n = sequence non complementary to the template region of telomerase RNA.